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Studies on Rhubarb (Rhei Rhizoma). II.[1] Anthraquinone Glycosides[2]

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Along with chrysophanol glucoside (I), mp 245—246°, $C_{21}H_{20}O_9 \cdot 1/2H_2O$, and 8-O-β-D-glucopyranosides of physcion, emodin, aloe-emodin and rhein, three new anthraquinone glucosides, II: mp 187—189°, $C_{21}H_{20}O_{10} \cdot 2H_2O$, III: mp 239—241°, $C_{21}H_{20}O_{10} \cdot 1/2H_2O$ and IV: mp 210—215° were isolated from the rhubarb, and II and III were respectively identified with 1,8-dihydroxy-3[-O-β-D-glucopyranosylmethyl]-aloe-emodin and 1-O-β-D-glucopyranosyl-emodin by spectral analyses of them, their permethylates and aglycones of permethylates.

Although physical constants of I were quite the same with those of chrysophanein (1-O-β-D-glucopyranosyl-chrysophanol), I was found to be a mixture of 1- and 8-O-β-D-glucopyranosyl-chrysophanols by nuclear magnetic resonance spectral analysis of the aglycone of its permethylate.

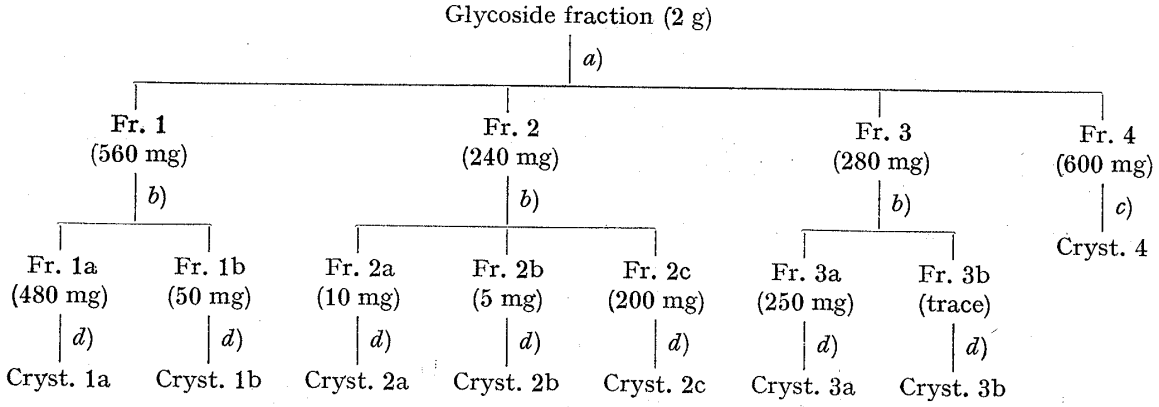
Since it has been known that the purgative effect of rhubarb is due to anthracene derivatives,[4] especially to their glycosides, chemical and pharmacological investigations have been the subject of numerous papers. On anthraquinone glycosides, which were considered to be the active principles at the beginning of the investigation, Hörhammer, *et al.*[5–7] extensively investigated and successfully isolated 1-O-β-D-glucoside of chrysophanol and 8-O-β-D-glucosides of physcion, emodin, aloe-emodin and rhein from *Rheum palmatum* Linnaeus *var. tanguticum* Maximowicz by polyamide column chromatography. They[5] also reported the presence of diglucosides of chrysophanol, aloe-emodin and rhein, however, their chemical structures have not been definitely established. Since the presence of sennosides A, B and C in rhubarb was reported by Zwaving[8] in 1965 and sennoside A was isolated from *Rheum coreanum* Nakai by Miyamoto, *et al.*[9] in 1967, dianthrone glycosides have been generally accepted as the principles accounting for the purging activity of rhubarb. Recently Miyamoto, *et al.*[10] isolated from rhubarb sennosides B, C, D and a new dianthrone glycoside, sennoside E, each having the equal biological activity with sennoside A.

In our preliminary work[11] on evaluation for the purgative activity of several kinds of rhubarb on mouse, we observed that the potency of the crude drug is in parallel to the content of sennoside A in every case, however, the chemically estimated content of sennoside A was much smaller than expected from ED_{50} values of sennoside A and the rhubarb itself.

1) Part I: A. Yagi, Y. Koizumi, and I. Nishioka, *Syôyakugaku Zasshi*, **25**, 52 (1971).
2) A part of this work was reported at the annual meeting of the Japanese Society of Pharmacognosy, Nishinomiya, October 1970.
3) Location: *a) Katakasu, Higashi-ku, Fukuoka; b) Tashiro, Tosu, Saga.*
4) J.W. Fairbairn, *Pharm. Weekblad*, **100**, 1493 (1965).
5) H. Wagner and L. Hörhammer, *Z. Naturforsch.*, **18b**, 89 (1963).
6) L. Hörhammer, H. Wagner, and E. Müller, *Chem. Ber.*, **98**, 2859 (1965).
7) L. Hörhammer, L. Farkas, H. Wagner, and E. Müller, *Chem. Ber.*, **97**, 1662 (1964).
8) J.H. Zwaving, *Planta Med.*, **13**, 474 (1965).
9) M. Miyamoto, S. Imai, M. Shinohara, S. Fujioka, M. Goto, T. Matsuoka, and H. Fujimura, *Yakugaku Zasshi*, **87**, 1040 (1967).
10) H. Oshio, S. Imai, S. Fujioka, T. Sugawara, M. Miyamoto, and M. Tsukui, *Chem. Pharm. Bull.* (Tokyo), **20**, 621 (1972).
11) H. Okabe, I. Nishioka, S. Tahara, H. Maejima, K. Matsuo, and N. Terashima, presented at the Annual Meeting of the Japanese Society of Pharmacognosy, Shizuoka, November, 1971.

This gap in the potency of rhubarb and that of sennoside A contained in it suggested the possible existence of some other material (or materials) having a potent purgative activity or a synergistic effect as observed in the case of senna,[12] and this discrepancy has prompted us to reinvestigate the glycosidal constituents of rhubarb. The authors have thoroughly reinvestigated the anthraquinone glycosidal constituents and this paper deals with unhomogeneity of chrysophanein (1-O-β-D-glucopyranosyl-chrysophanol) and with isolation and structure elucidation of 1,8-dihydroxy-3[-O-β-D-glucopyranosylmethyl-]-anthraquinone (ω-O-β-D-glucopyranosyl-aloe-emodin) and 1-O-β-D-glucopyranosyl-emodin.

The anthraquinone glycoside fraction obtained from Chinese rhubarb[13] by the procedure shown in Chart 1 exhibited on thin-layer chromatogram (TLC) (Fig. 1A) at least five spots of anthraquinone glycosides and it was column-chromatographed over silica gel with solvent AcOEt–MeOH–H_2O (90: 5: 5, v/v) to give four fractions (Fr. 1—4), among which Fr. 4 showed a single spot on polyamide TLC (solvent; MeOH) and it was recrystallized from 70% acetone to give yellow needles (Cryst. 4), mp 285—289°, $C_{21}H_{18}O_{11}$· Fr. 1 and 3 gave two spots respectively, while Fr. 2 gave three spots on polyamide TLC (Fig. 1B), and they were separated by means of polyamide column chromatography to give thin-layer chromatographically



a) silica gel column chromatography, solvent: AcOEt–MeOH–H_2O (90: 5: 5)
b) polyamide column chromatography, solvent: MeOH
c) recrystallization from 70% acetone
d) recrystallization from MeOH

Chart 1. Isolation of Anthraquinone Glycosides

TABLE I. *Rf* Values and Colors of Anthraquinone Glycosides

	Rf value		Color of the spot on Kieselgel G plate		
	I[a]	II[b]	Day-light	UV	10% KOH
Cryst. 1a	0.52	0.99	yellow	orange	reddish orange
Cryst. 1b	0.52	0.93	yellow	orange	reddish orange
Cryst. 2a	0.48	0.92	orange yellow	orange	violet
Cryst. 2b	0.46	0.66	orange yellow	dark violet	reddish violet
Cryst. 2c	0.46	0.55	orange yellow	dark violet	reddish violet
Cryst. 3a	0.39	0.98	yellow	orange yellow	reddlsh orange
Cryst. 3b	0.38	0.55	yellow	dark violet	reddish violet
Cryst. 4	0.10	0.09	yellow	orange	reddish orange

a) AcOEt–MeOH–H_2O (100: 16.5: 13.5)/Kieselgel G
b) MeOH/Polyamide B-10

12) J.W. Fairbairn and M.R.I. Saleh, *Nature*, **167**, 988 (1951).
13) "Tō-gai Daiō" purchased in the Chinese market.
14) L. Hörhammer, H. Wagner, and G. Bittner, *Pharm. Ztg. Ver. Apotheker-Ztg.*, **108**, 259 (1963).

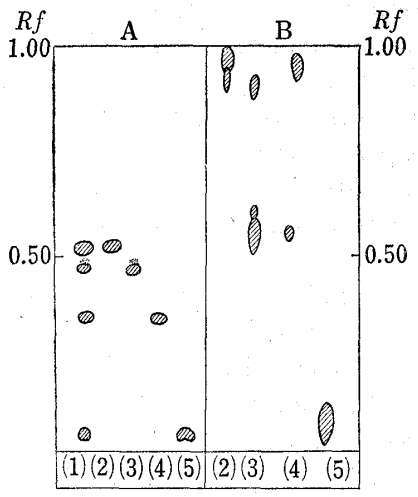


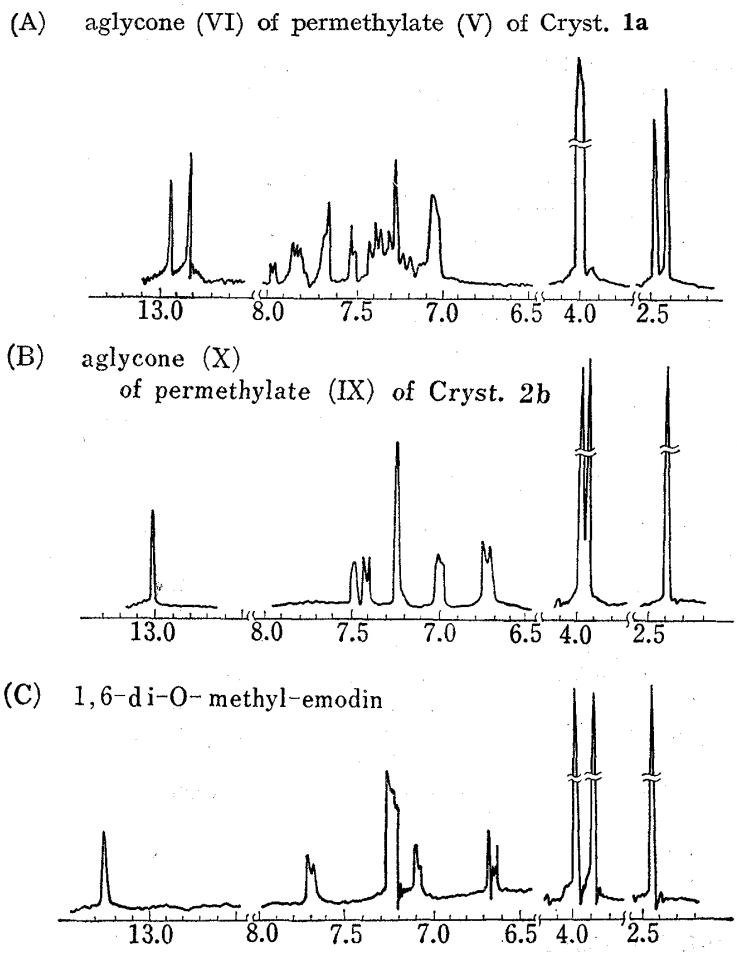
Fig. 1. Thin-Layer Chromatograms of Glycoside Fractions

A: AcOEt–MeOH–H_2O (100:16.5:13.5)[14]/ Kieselgel G
B: MeOH/polyamide B-10
(1) glycoside fraction
(2) Fr. 1, (3) Fr. 2, (4) Fr. 3, (5) Fr. 4

homogeneous fractions (Fr. 1a, b; Fr. 2a, b, c; Fr. 3a, b) and each fraction was recrystallized from methanol to give Cryst. 1a (I), yellow needles, mp 245—246°, $C_{21}H_{20}O_9 \cdot 1/2H_2O$; Cryst. 1b, orange needles, mp 237—239°, $C_{22}H_{22}O_{10} \cdot 1/2H_2O$; Cryst. 2a (II), orange needles, mp 187—189°, $C_{21}H_{20}O_{10} \cdot 2H_2O$; Cryst. 2b (III), orange needles, mp 239—241°, $C_{21}H_{20}O_{10} \cdot 1/2H_2O$; Cryst. 2c, orange needles, mp 189—190°, $C_{21}H_{20}O_{10} \cdot 1/2H_2O$; Cryst. 3a, orange needles, mp 239—240°, $C_{21}H_{20}O_{10}$; and Cryst. 3b (IV), orange needles, mp 210—215°.

Cryst. 1b, 2c, 3a and 4 were respectively identified with 8-O-β-D-glucopyranosides of physcion, emodin, aloe-emodin and rhein by the comparison of their physical constants with those reported,[5–7] the examination of their aglycones and sugars, and moreover, by the nuclear magnetic resonance (NMR) spectral analysis of aglycones of their permethylates.

Cryst. 1a (I) gave chrysophanol and D-glucose on acid hydrolysis and its physical constants were quite consistent with those of chrysophanein (1-O-β-D-glucopyranosyl-chrysophanol) reported by Hörhammer, *et al.*[5,6] and the NMR spectrum of the permethylate (V) of Cryst. 1a depicted a doublet (J=6.8 Hz) for the anomeric proton of the



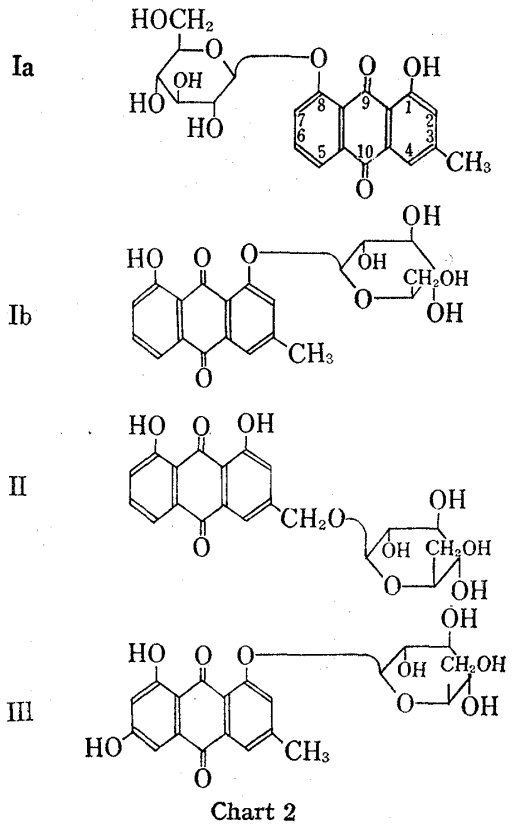


Chart 2

Fig. 2. NMR Spectra of Aglycones of Permethylated Glycosides (60 MHz, $CDCl_3$, TMS)

sugar moiety at 5.00 ppm showing the glucopyranosyl group in Cryst. 1a to be in β-configuration. However, the NMR spectrum (Fig. 2A) of the aglycone (VI) obtained by methanolysis of the permethylate (V) depicted two singlets (2.42 and 2.47 ppm, intensity ratio, 5: 3) accounting for three protons of the C_3-methyl group and two singlets at 12.82 and 12.95 ppm accounting for one proton of the chelated hydroxyl group, and this NMR spectrum was almost the same with that of the mixture of 1- and 8-O-methyl-chrysophanols prepared by partial methylation of chrysophanol. This suggested that VI is the mixture of 1- and 8-O-methyl- chrysophanols and thus, Cryst. 1a is the mixture of 8-O-β-D-glucopyranoside (Ia) and 1-O-β-D-glucopyranoside (Ib) of chrysophanol in a molar ratio of 5: 3 (or 3: 5). Cryst. 1a showed a single spot on TLC and attempted separation of them and their acetates or benzoates could not be achieved.

Cryst. 2a (II) yielded aloe-emodin and D-glucose on acid hydrolysis and when methylated byHakomori's method,[15] it gave a permethylate (VII), mp 134°, $C_{27}H_{32}O_{10}\cdot 1/2H_2O$. Its NMR spectrum depicted a doublet (4.88 ppm, J=7.2 Hz) for the anomeric proton indicating the permethylated D-glucosyl group to be in β-configuration. Infrared (IR) spectrum of the aglycone (VIII) showed peaks for the non-chelated carbonyl group at 1655 cm^{-1} and the hydroxyl group at 3480 cm^{-1}, and there was no peak of the chelated carbonyl group. Its ultraviolet (UV) spectrum exhibited the absorption maxima at 223, 257 and 392 mμ and they showed no bathochromic shifts on addition of alkali. These spectral data indicated VIII to be 1,8-di-O-methyl-aloe-emodin and the identity was proved by the direct comparison of VIII with 1,8-di-O-methyl-aloe-emodin prepared by methylation of aloe-emodin with diazomethane. Accordingly, Cryst. 2a was concluded to be 1,8-dihydroxy-3-[O-β-D-glucopyranosylmethyl]-anthraquinone (ω-O-β-D-glucopyranosyl-aloe-emodin).

Cryst. 2b (III) gave emodin and D-glucose on acid hydrolysis and when methylated, it gave a permethylate (IX), mp 194—196°, $C_{27}H_{32}O_{10}\cdot 1/2H_2O$. Its NMR spectrum depicted a doublet (4.99 ppm, J=7.2 Hz) for the anomeric proton indicating the permethylated D-glucopyranosyl group to be β-linked. When methanolized, IX gave emodin di-O-methylether (X). Its IR spectrum showed the peak for the chelated carbonyl group at 1635 cm^{-1} in addition to the non-chelated carbonyl group at 1670 cm^{-1}, and its NMR spectrum (Fig. 2B) exhibited a singlet for the C_3-methyl protons at 2.40 ppm and a singlet for the hydroxyl proton chelated with C_9-carbonyl group at 13.02 ppm. 1,6-Di-O-methyl-emodin obtained from 8-O-β-D-glucopyranosyl-emodin showed a singlet for the C_3-methyl protons at 2.47 ppm and a singlet for the chelated C_8-hydroxyl proton at 13.26 ppm (Fig. 2C). The mixture of 1,6- and 6,8-di-O-methyl-emodins, prepared by methylation of emodin with silver oxide and methyl iodide, showed on the NMR spectrum, two singlets for C_3-methyl protons at 2.40 and 2.47 ppm and two singlets due to chelated hydroxyl protons at 13.02 and 13.26 ppm. These NMR spectral data supported that X is 6,8-di-O-methyl-emodin. Thus, Cryst. 2b was concluded to be 1-O-β-D-glucopyranosyl-emodin.[16] This is the first 1-O-glucopyranosyl-anthraquinone isolated from higher plants.

Cryst. 3b (IV) gave D-glucose and the aglycone having the same *Rf* value with that of aloe-emodin on TLC, but no further investigation on its structure could not be carried out because of its extremely low yield.

As mentioned above, the presence of 1-O-β-D-glucosides of chrysophanol and emodin was determined, however, against our sincere expectation, corresponding glucosides of physcion, aloe-emodin and rhein could not be detected. This raised interesting problems regarding both the step of biogenetic glucosylation in the course of oxidation of C_3-methyl group in

15) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 255 (1964).

16) Steglich, *et al.*[17] cited the NMR spectrum of 1-O-β-D-glucopyranosyl-emodin peracetate in their report on determination of the position of O-alkyl or O-glycosyl substituents in 1,8-dihydroxyanthraquinone derivatives, and noted that the original compound was isolated from *Dermocibe sanguinea*, but details are not published yet.

17) W. Steglich and W. Lösel, *Tetrahedron*, **25**, 4391 (1969).

chrysophanol or its precursor to hydroxymethyl and carboxylic groups and the influence of C_3-substituents on the position of glucosylation.

The purgative activities of these newly isolated anthraquinone glucosides are planned to be determined.

Table II. *Rf* Values of Aglycones

	Rf value	
	I[a]	II[b]
Aglycone of Cryst. 1a	0.95	0.42
Crystl 1b	0.95	0.34
Cryst. 2a	0.43	
Cryst. 2b	0.60	
Cryst. 2c	0.60	
Cryst. 3a	0.43	
Cryst. 3b	0.43	
Cryst. 4	0.27	
Chrysophanol	0.42	0.95
Physcion	0.95	0.34
Emodin	0.60	
Aloe-emodin	0.43	
Rhein	0.27	

a) benzene–HCOOEt–AcOEt–HCOOH (75: 24: 0.8: 0.2)/Kieselgel G
b) benzene–hexane (1: 1)/Kieselgel G

Experimental[18]

Extraction and Isolation of Anthraquinone Glycosides (Chart 1)——Powdered rhubarb (1 kg) was percolated with 60% MeOH (6 liters in total) and the 60% MeOH solution was concentrated to 1/3 volume and then adjusted at pH 3 adding citric acid. The acidic solution was shaken with BuOH (1.5 liters × 3), BuOH layer was separated and then BuOH was evaporated *in vacuo* to give a dark brown resinous residue (102 g). The BuOH extract was washed with ether (300 ml) to remove free anthraquinones and ether–insoluble portion was washed with acetone (500 ml) to give a light orange yellow acetone–insoluble powder (28 g) of the glycoside fraction. The glycoside graction was fractionated first by silica gel column chromatography to four fractions (Fr. 1—4) (Fig. 1A), among which Fr. 1, 2 and 3 were respectively fractionated by polyamide column chromatography. Thin–layer chromatographically homogeneous glycosidic fractions obtained were respectively recrystallized from 70% acetone or MeOH. In column chromatography, three hundred fold of silica gel, hundred fold of polyamide were used.

Cryst. 1a (Mixture of 1- and 8-O-β-D-glucopyranosyl-chrysophanols) (I): Yellow needles (MeOH). mp 245—246°, IR ν_{max}^{KBr} cm^{-1}: 1640 (chelated C=O), 1673 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 222 (4.45), 283 (4.19), 412 (3.96). *Anal.* Calcd. for $C_{21}H_{20}O_9 \cdot 1/2H_2O$: C, 59.30; H, 4.94. Found: C, 59.30; H, 4.91.

Cryst. 2a (1,8-Dihydroxy-3-[-O-β-D-glucopyranosylmethyl]-anthraquinone) (ω-O-β-D-glucopyranosyl-aloe-emodin) (II): Orange needles (MeOH). mp 187—189°. IR ν_{max}^{KBr} cm^{-1}: 1626 (chelated C=O), 1674 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 226 (4.72), 256 (4.40), 433 (4.05). *Anal.* Calcd. for $C_{21}H_{20}O_{10} \cdot 2H_2O$: C, 53.84; H, 5.12. Found: C, 54.22; H, 4.86.

18) Melting points were determined on a Kofler hot plate and on a Yanagimoto micro melting point apparatus, and are uncorrected. NMR spectra were taken at 60 MHz on a JEOL-JNM-C-60H spectrometer in $CDCl_3$ using Me_4Si as internal reference and chemical shifts are shown in δ-scale (s; singlet, d; doublet). IR spectra were obtained with Nippon Bunko DS-301 and DS-701G spectrometers, and UV spectra with a Shimadzu SV-50A spectrophotometer, and mass spectra with a JEOL-JNM-01SG spectrometer provided with glass inlet system heated at 80—120°, ion accelerating voltage, ionizing voltage, ionizing current being 6.3 kV, 75 eV and 200 μA, respectively. TLC was conducted on Kieselgel G nach Stahl and Polyamide B-10 (Wako). In column chromatography, Kieselgel (E. Merck) and Polyamide C-200 (Wako) were employed. PC was conducted by the ascending method on Toyo roshi No. 50 filter papers.

Cryst. 2b (1-O-β-D-Glucopyranosyl-emodin) (III): Orange needles (MeOH). mp 239—241°. IR ν_{max}^{KBr} cm^{-1}: 1625 (chelated C=O), 1660 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 253 (4.34), 287 (4.36), 425 (3.92). *Anal.* Calcd. for $C_{21}H_{20}O_{10}\cdot 1/2H_2O$: C, 57.14; H, 4.79. Found: C, 57.37; H, 4.79.

Cryst. 3b (IV): Orange needles (MeOH), mp 210—215°. UV λ_{max}^{MeOH} mμ: 222, 270, 285, 425.

Identification of Component Sugars and Aglycones——About 3 mg each of anthraquinone glycosides was heated at around 90° in 0.5 ml of 10% aqueous HCl for 1 hr. The reaction mixture was diluted with 5 ml of MeOH, neutralized with Ag_2O and evaporated. The residue was dissolved in a minimum volume of MeOH (*ca.* 0.5 ml) and the aglycone deposited on cooling was filtered and checked by TLC (Table II). The filtrate was concentrated and submitted to PC. All component sugars of glycosides respectively showed the spot with the same *Rf* values with those of D-glucose (*Rf* 0.16 by BuOH–AcOH–H_2O (4: 1: 5, top layer), 0.57 by BuOH–pyridine–H_2O (6: 2: 3, top layer)+pyridine (1), triple run).

Permethylation of Anthraquinone Glucosides and Methanolysis of Permethylates——About 30 mg of the glycoside was methylated by the Hakomori's method[15] and purified by column chromatography over silica gel (*ca.* 6 g) eluted with hexane followed by $CHCl_3$. The $CHCl_3$ eluate was recrystallized from MeOH to give TLC homogeneous permethylate (*ca.* 20 mg). The permethylate was heated at reflux in 2N-methanolic HCl (1 ml) for 1 hr and the aglycone crystallized on cooling was filtered and recrystallized. The filtrate was concentrated and checked by TLC on Kieselgel G (solvent: 1% MeOH in AcOEt). All component methylated sugars of permethylates respectively gave the spot with the same *Rf* value (*Rf* 0.52) with that of methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside.

Permethylate (V) of Cryst. 1a: Light yellow needles (MeOH). mp 193—195°. IR ν_{max}^{KBr} cm^{-1}: 1670 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 221 (4.51), 258 (4.44), 385 (3.89). NMR (ppm): 5.00 (1H, d, J=6.8 Hz). *Anal.* Calcd. for $C_{26}H_{30}O_9$: C, 64.18; H, 6.22. Found: C, 64.09; H, 6.37. Aglycone (VI) of V: Yellow needles (MeOH). mp 165—170°. IR ν_{max}^{KBr} cm^{-1}: 1640 (chelated C=O), 1672 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 224 (4.66), 259 (4.39), 283 (shoulder) (4.16), 415 (4.00); $\lambda_{max}^{1\%\ KOH\ in\ MeOH}$ mμ: 250, 280, 319, 490. NMR (ppm): 2.42 (1.9H, s), 2.47 (1.1H, s), 4.02, 4.04 (3H, in total, singlets), 12.82 (0.63H, s), 12.95 (0.37H, s). Mass Spectrum: [M$^+$], Calcd. for $C_{16}H_{12}O_4$: 268.074. Found: 268.076.

Permethylate (VII) of Cryst. 2a: Pale yellow needles (hexane). mp 134°. IR ν_{max}^{KBr} cm^{-1}: 1673 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 224 (4.68), 257 (4.41), 275 (shoulder) (4.25), 390 (3.95). NMR (ppm): 4.88 (1H, d, J=7.2 Hz). *Anal.* Calcd. for $C_{27}H_{32}O_{10}\cdot 1/2H_2O$: C, 61.71; H, 6.28. Found: C, 61.99; H, 6.34. Aglycone (VIII) of VII: Pale yellow needles (MeOH). mp 229—231°. IR ν_{max}^{KBr} cm^{-1}: 1655 (non-chelated C=O), 3480 (OH). UV λ_{max}^{MeOH} mμ (log ε): 223 (4.60), 257 (4.34), 392 (3.87); $\lambda_{max}^{1\%\ KOH\ in\ MeOH}$ mμ: 225, 257, 392. Mass Spectrum: [M$^+$], Calcd. for $C_{17}H_{14}O_5$: 298.084. Found: C, 298.085.

Permethylate (IX) of Cryst. 2b: Yellow needles (MeOH). mp 194—196°. IR ν_{max}^{KBr} cm^{-1}: 1676 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 221 (4.63), 277 (4.39). 400 (3.78). NMR (ppm): 4.98 (1H, d, J= 6.8 Hz). *Anal.* Calcd. for $C_{27}H_{32}O_{10}\cdot 1/2H_2O$: C, 61.71; H, 6.28. Found: C, 62.02; H, 6.32. Aglycone (X) of IX: Orange needles (MeOH). mp 208—208.5°. IR ν_{max}^{KBr} cm^{-1}: 1634 (chelated C=O), 1670 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 224.5 (4.25), 246 (3.77), 269 (3.97), 280 (3.97), 425 (3.63); $\lambda_{max}^{1\%\ KOH\ in\ MeOH}$ mμ: 265, 296, 490. NMR (ppm): 2.40 (3H, s, -C$\underline{H}_3$), 3.95 (3H, s, -OC$\underline{H}_3$), 4.00 (3H, s, -OC$\underline{H}_3$), 6.73 (1H,d, J=2.3 Hz, aromatic H), 7.05 (1H, disturbed d, aromatic H), 7.40 (1H, d, J=2.3 Hz, aromatic H), 7.52 (1H, disturbed d, aromatic H), 13.02 (1H, s, -OH). Mass Spectrum: [M$^+$], Calcd. for $C_{17}H_{14}O_5$: 298.084. Found: 298.080.

Partial Methylation of Chrysophanol and Emodin——To the solution of the anthraquinone (*ca.* 150 mg) in CH_3I (3 ml) was added Ag_2O (1 g) and the mixture was stirred for 1 hr at room temperature. Ag_2O was filtered off and CH_3I was evaporated. The resulting reddish orange powder was purified by silica gel column chromatography (silica gel; *ca.* 15 g, solvent; benzene–AcOEt), and recrystallized from MeOH.

Mixture of 1- and 8-O-Methyl-chrysophanols: Orange crystalline powder. mp 178—182°. IR ν_{max}^{KBr} cm^{-1}: 1640 (chelated C=O), 1670 (non-chelated C=O). UV λ_{max}^{MeOH} mμ: 224, 258, 283 (shoulder), 415; $\lambda_{max}^{1\%\ KOH\ in\ MeOH}$ mμ: 250, 280 (shoulder), 314, 490. NMR (ppm): 2.42 (1.5H, s), 2.47 (1.5H, s), 4.02, 4.04 (3H, in total, singlets), 12.82 (0.5H, s), 12.95 (0.5H, s). *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.63; H, 4.51. Found: C, 71.16; H, 4.64.

Mixture of 6,8- and 1,6-Di-O-methyl-emodins: Orange fine plates (MeOH). mp 200—204°. IR ν_{max}^{KBr} cm^{-1}: 1630 (chelated C=O), 1666 (non-chelated C=O). UV λ_{max}^{MeOH} mμ: 224, 268, 282, 425; $\lambda_{max}^{1\%\ KOH\ in\ MeOH}$ mμ: 257, 296, 490. NMR (ppm): 2.40, 2.47 (3H in total, singlets), 3.38, 3.98, 4.00 (6H in total, singlets), 13.02 (0.6H, s), 13.26 (0.4H, s). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 67.91; H, 4.86.

1,6-Di-O-methyl-emodin——Prepared by methanolysis of 8-O-β-D-glucopyranosyl-emodin permethylate. Orange needles (MeOH). mp 201—202°. IR ν_{max}^{KBr} cm^{-1}: 1632 (chelated C=O), 1670 (non-chelated C=O). UV λ_{max}^{MeOH} mμ (log ε): 222 (4.25), 250 (3.93), 265 (3.96), 283 (4.00), 425 (3.68); $\lambda_{max}^{1\%\ KOH\ in\ MeOH}$ mμ: 257, 296, 490. NMR (ppm): 2.47 (3H, s), 3.90 (3H, s, -OC$\underline{H}_3$), 4.02 (3H, s, -OC$\underline{H}_3$), 13.26 (1H, s, -O$\underline{H}$). Mass Spectrum: [M$^+$], Calcd. for $C_{17}H_{14}O_5$: 298.084. Found: 298.081.

1,8-Di-O-methyl-aloe-emodin——Prepared by methylation of aloe-emodin with CH_2N_2 and purified by silica gel column chromatography (solvent: $CHCl_3$). Pale yellow needles (MeOH). mp 229—231°.

UV λ_{max}^{MeOH} mμ (log ε): 223 (4.56), 258 (4.31), 275 (shoulder) (4.06), 390 (3.85); UV $\lambda_{max}^{1\% KOH in MeOH}$ mμ: 223, 258, 390. Mass Spectrum: [M+], Calcd. for $C_{17}H_{14}O_5$: 298.084. Found: 298.086.

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